DECLARATION

I, Toshiyuki Ueno, a member of Yokogawa Electric Corporation (Yokogawa Denki Kabushiki Gaisha) having a principal place of business at 9-32, Nakacho 2-chome, Musashino-shi, Tokyo 180-8750 Japan, do solemnly declare that the attached documents are full, true and faithful translation made by me this 30th day of June 2008 of a certified copy of the Japanese Patent Application No. 1999-149400 "Electrophoresis System" consisting of Application for certificate dully certified thereon and Specification. And I make this solemn declaration conscientiously believing the same to be true.

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[Document Name] Specification

[Title of the Invention] Electrophoresis System

[What Is Claimed Is]

[Claim 1]

An electrophoresis system for conducting electrophoresis of a sample marked with fluorescent coloring matter in a lane area so that a fluorescence pattern that is produced is read, comprising:

an electrophoresis unit for conducting electrophoresis by flowing a plurality of samples prepared by combining various types of target substance such as protein or DNA with different types of fluorescent coloring matter into the same lane of said lane area; and

a confocal scanner or a fluorescence imaging system wherein samples in said lane area are scanned with excitation light and polychrome fluorescence patterns of said samples produced by irradiating said excitation light are simultaneously detected through a plurality of filters having different transmission characteristics,

whereby a plurality of electrophoretic patterns are detected simultaneously.

[Claim 2]

A three-dimensional electrophoresis system for conducting electrophoresis of a sample marked with fluorescent coloring matter in a lane area so that a fluorescence pattern that is produced is read, comprising:

an electrophoresis unit for conducting electrophoresis by flowing various types of target substance such as protein or DNA into said lane area and applying a gradient of physical quantities such as voltage, pH, density or concentration in the direction of depth of said sample; and

a scanning confocal microscope, a non-scanning confocal microscope, or a dual-grating excitation microscope, which is configured so that a sample in said lane area is scanned with excitation light and a fluorescence pattern of said sample produced by irradiating said excitation light is detected,

whereby the three-dimensional position and concentration of said sample are detected.

[Claim 3]

A three-dimensional polychrome electrophoresis system as defined in claim 2, wherein different physical gradients are applied to said electrophoresis unit of said electrophoresis system in two horizontal directions and in one vertical direction so that sample separation is performed simultaneously on all three axes.

[Claim 4]

A three-dimensional electrophoresis system as defined in claim 2, wherein samples and markers are placed in the depth direction in said electrophoresis unit.

[Claim 5]

A three-dimensional electrophoresis system as defined in claim 2, wherein each aperture of said non-scanning confocal microscope shares the same position with each sample or is positioned inside each sample.

[Claim 6]

An electrophoresis system as defined in claim 1 or 2, wherein said non-scanning confocal microscope further comprises beam-condensing means on the light-source side of said aperture.

[Claim 7]

An electrophoresis system as defined in claim 2, wherein the distribution of density in the depth direction is realized by wetting only one side of a gel with a highly concentrated solution, applying a density gradient in the depth direction by means of centrifugation, or stacking multiple layers of gel with different concentrations.

[Detailed Description of the Invention]

[0001]

[Technical Field to Which the Invention Pertains]

The present invention relates to an electrophoresis system used in the field of bioengineering. More specifically, the invention relates to improvements made in order to increase the operating speed and resolution of the electrophoresis system.

[0002]

[Description of the Prior Art]

Electrophoresis methods have been well known as means for analyzing the structure of genes or proteins, such as amino acid, using an inexpensive, simple system. The methods are very often used in the field of bioengineering.

[0003]

These electrophoresis methods include a disk electrophoresis method using polyacrylamide, an SDS (sodium dodecyl sulfate) polyacrylamide-gel electrophoresis method, an isoelectric-point electrophoresis method, a nucleic-acid gel electrophoresis method, an electrophoresis method based on the effects of interaction with other molecules, a two-dimensional electrophoresis method, and a capillary electrophoresis method.

[0004]

FIG. 9 shows an example of a conventional electrophoresis measurement system. The system consists mainly of two components, an electrophoresis unit 10 and a signal processor 20.

The electrophoresis unit 10 consists of a lane area 11, a first electrode 12 and a second electrode 13 for applying voltage to the lane area 11, a support plate 14 for supporting the lane area 11 and the first

and second electrodes 12 and 13, a power unit 15 for electrophoresis used to supply voltage to the two electrodes, a light source 16 for emitting light to excite a fluorescent substance, an optical fiber 17 for guiding light emitted by the light source 16, and an optical detector 18 for condensing fluorescent light produced by a fluorescent substance to convert the light to an electric signal after selectively introducing light of a specific wavelength through an optical filter.

[0005]

The signal processor 20 is designed to be able to receive an electric signal from the optical detector 18 to perform appropriate processes, such as converting the electric signal to digital data or performing preliminary processes, including summing and averaging. The output of the signal processor 20 is sent to a data processor, which is not shown in FIG. 4, where samples are submitted to an analysis process for examination.

[0006]

In such a measurement system as described above, electrophoresis begins when a gel is injected into the lane area 11, samples of DNA segments marked with a fluorescent substance are injected from above the gel, and voltage is applied to the first and second electrodes 12 and 13 using the power unit 15. Molecules contained in the samples gather in each lane of samples as classified by molecular weight, each group of molecules forming a band. Since molecules with lower molecular weight have higher speeds of electrophoresis, they migrate longer distances within the same length of time.

[0007]

These bands are detected by irradiating the gel with laser light, for example, emitted by the light source 16, causing marks of the fluorescent substance that concentrate on the bands in the gel to emit fluorescent light, and detecting the fluorescent light with the optical detector 18.

[0008]

That is, if the gel is irradiated with laser light, the fluorescent substance within part of the gel, which exists along a line 31 shown in FIG. 10, is excited to emit fluorescent light. This fluorescent light is detected at a given position of each lane, as it is searched for in the direction of electrophoresis with the lapse of time. Consequently, the fluorescent light is detected when a band 32 of each lane crosses the line 31. Thus, it is possible to obtain a signal representing the intensity pattern of fluorescence for a single lane.

The data processor, which is not shown in FIG. 10, is designed to be able to analyze each base sequence of DNA from the pattern signal.

[0009]

[Problems to Be Solved by the Invention]

Such a conventional electrophoresis system as described above has had the following problems, however:

[1] A prolonged period of time is required to perform measurement.

[2] The separability is not sufficient; too many lanes are required to separate a variety of DNA segments. Furthermore, information on the correlation among three or more dimensions is not available since the system is limited to two-dimensional analysis.

[3] The system requires a large installation space. For example, the lane area is as large as 50 cm × 50 cm or 5 cm × 5 cm.

[4] A two-dimensional system is particularly inferior in terms of positional reproducibility. This problem may be solved by applying markers to other lanes and then referencing them. However, applying markers in this way increases the lane area.

[0010]

The object of the present invention is to solve the aforementioned problems by providing an electrophoresis system which has a compact lane area, offers highly accurate electrophoretic patterns, and permits faster acquisition of large amounts of interrelated information.

[0011]

[Means for Solving Problems]

In order to achieve the aforementioned object, the present invention provides, as defined in claim 1, an electrophoresis system for conducting electrophoresis of a sample marked with fluorescent coloring matter in a lane area so that a fluorescence pattern that is produced is read, comprising:

an electrophoresis unit for conducting electrophoresis by flowing a plurality of samples prepared by combining various types of target substance such as protein or DNA with different types of fluorescent coloring matter into the same lane of the lane area; and

a confocal scanner or a fluorescence imaging system wherein samples in the lane area are scanned with excitation light and polychrome

fluorescence patterns of the samples produced by irradiating the excitation light are simultaneously detected through a plurality of filters having different transmission characteristics,

thereby allowing a plurality of electrophoretic patterns to be detected simultaneously.

[0012]

According to this configuration, it is possible to reduce the number of lanes and thereby the size of the lane area, prevent the voltage gradient and the gel from becoming uneven, and perform precise measurement.

Furthermore, it is possible to simultaneously detect polychrome fluorescence patterns using the confocal scanner or fluorescence imaging system and thereby reduce the time required for detection.

[0013]

According to claim 2, the present invention provides an electrophoresis system for conducting electrophoresis of a sample marked with fluorescent coloring matter in a lane area so that a fluorescence pattern that is produced is read, comprising:

an electrophoresis unit for conducting electrophoresis by flowing various types of target substance such as protein or DNA into the lane area and applying a gradient of physical quantities such as voltage, pH, density or concentration in the direction of depth of the sample; and

a scanning confocal microscope, a non-scanning confocal microscope, or a dual-grating excitation microscope, which is configured so that a sample in the lane area is scanned with excitation light and a

fluorescence pattern of the sample produced by irradiating the excitation light is detected,

thereby allowing the three-dimensional position and concentration of the sample to be detected.

[0014]

According to the configuration described above, it is possible to detect three-dimensional electrophoresis patterns simultaneously by using a confocal microscope or a dual-grating microscope even in a case where electrophoresis is conducted by applying a gradient of physical quantities, such as voltage, pH, density or concentration, in the direction of depth of the sample.

[0015]

According to claim 3, the electrophoresis system as defined in claim 2 has a feature wherein different physical gradients are applied in two horizontal directions and in one vertical direction and electrophoresis is conducted along all these three axes simultaneously, thereby allowing both high-speed measurement of samples and measurement of the relationship of mutual influence among the three axes.

[0016]

According to claim 4, samples and markers are placed in the depth direction of the samples so that the samples share the same gel concentration, temperature difference, voltage distribution and other conditions, thereby enabling precise measurement with a compact system.

[0017]

According to claim 5, each aperture of the non-scanning confocal

microscope shares the same position with each sample or is positioned inside each sample, thereby enabling measurement with higher S/N ratios and without any adverse effect that may result when edges of the sample are measured.

[0018]

According to claim 6, the non-scanning confocal microscope further comprises beam-condensing means on the light-source side of the aperture, thereby enabling effective use of the light source.

[0019]

According to claim 7, the distribution of density in the depth direction can be realized by wetting only one side of a gel with a highly concentrated solution, applying a density gradient in the depth direction by means of centrifugation, or stacking multiple layers of gel with different concentrations.

[0020]

[Mode for Carrying out the Invention]

The present invention is described in detail below with reference to the accompanying drawings. FIG. 1 is a schematic block diagram showing the features of one embodiment of an electrophoresis system in accordance with the present invention. In FIG. 1, the numeral 100 indicates a confocal microscope and the numeral 200 indicates an electrophoresis unit.

[0021]

The confocal microscope 100 (also referred to as the confocal optical scanner 100) is designed to be able to optically scan the gel in a lane

area 201 and read the electrophoretic patterns of fluorescent light emitted from the gel. The confocal microscope 100 is configured as described below.

[0022]

Excitation light (blue laser light with a wavelength of λ1, for example) emitted by a light source 101 is made parallel by a lens 102, is reflected by a dichroic mirror 103, and is condensed onto the slits of a slit array 105 through a lens 104. Excitation light that has passed through the slits is narrowed by an objective lens 106 and enters the gel in the lane area 201. The fluorescent substance in the lane area 201 is excited by this light and emits fluorescent light.

[0023]

The fluorescent light thus produced retraces the path that the excitation light followed, by travelling through the objective lens 106 to the slit array 105 and the lens 104. It then passes through the dichroic mirror 103 to reach another dichroic mirror 107.

It should be noted that the dichroic mirror 103 reflects light with a wavelength of λ1 (blue, for example) and allows light with wavelengths greater than λ1 to pass through it. Likewise, the dichroic mirror 107 reflects light with a wavelength of λ2 (green, for example) and allows light with a wavelength of λ3 (red, for example) to pass through it. The relationship among λ1, λ2 and λ3 is shown in FIG. 2.

[0024]

The light with a wavelength of λ2 that has been reflected by the dichroic mirror 107 is condensed onto an optical detector 109 through a lens 108. On the other hand, the light with a wavelength of λ3 that has

passed through the dichroic mirror 107 is condensed onto an optical detector 111 through a lens 110.

If the slit array 105 is moved and controlled in such a manner that light emitted by the light source 101 scans across the surface of the lane area 201, the electrophoretic pattern of fluorescence produced in the lane area 201 is formed at each of the optical detectors 109 and 111.

[0025]

At this point, only the electrophoretic pattern of green fluorescence is formed at the optical detector 109, whereas only the electrophoretic pattern of red fluorescence is formed at the optical detector 111. The optical detectors 109 and 111 convert these images to electric signals and output them.

[0026]

The electrophoresis unit 200 is equipped with the lane area 201 and power unit 202 for supplying voltage to cause electrophoresis in the lane area 201.

[0027]

As described above, using a confocal optical scanner makes it possible to easily and precisely measure the polychrome electrophoretic pattern of fluorescence produced in the lane area 201.

[0028]

It is not possible, however, to determine the absolute value of molecular weight by electrophoresis. Therefore, under normal conditions, reference marker molecules are flowed into neighboring lanes, as shown in FIG. 3. This method is problematic since it requires more space and

involves measurement errors due to the difficulty in applying voltage evenly to all of the lanes.

[0029]

In the present invention, a sample is flowed together with a reference marker molecule (hereinafter simply referred to as a "marker") into the same lane, as shown in FIG. 4. At this point, coloring matters with different fluorescence wavelengths are combined with the respective markers and samples. A material thus prepared is submitted to electrophoresis and scanned with the confocal optical scanner. Consequently, it is possible to detect two or more electrophoretic patterns of fluorescence at the same time.

[0030]

FIG. 5 is another embodiment of the present invention. Unlike the widely known two-dimensional electrophoresis, the embodiment of FIG. 5 is an example of three-dimensional electrophoresis in which another dimension is added in the direction of depth (Z-axis direction).

[0031]

In this example, methods for applying a voltage gradient and a pH gradient in the X-axis (longitudinal), Y-axis (lateral) and Z-axis (depth) directions include:

1) applying high voltage in the X-axis direction, pH gradient in the Y-axis direction and low voltage in the Z-axis direction;
2) applying voltage in the X-axis direction, pH gradient in the Y-axis direction and multi-layer gel with each layer having a different concentration in the Z-axis direction; and

3) applying voltage in the X-axis direction, pH gradient in the Y-axis direction and a voltage gradient in the Z-axis direction, in order to perform affinity electrophoresis.

[0032]

In this example, the electrophoresis system is configured so that the optically scanned surface of the lane area 201 can be moved up and down along the optical axis (in the Z-axis direction). For example, the electrophoresis system is configured so that the objective lens 106 of the confocal optical scanner 100 can be moved up and down. Then, X-Y axis polychrome electrophoretic patterns of fluorescence are detected by controlling the optically scanned surface in the Z-axis direction. Consequently, it is possible to easily acquire three-dimensional information.

[0033]

In the explanation given above, only specific preferred embodiments are mentioned for the purpose of describing the present invention and showing examples of carrying out the invention. The above-mentioned embodiments are therefore to be considered as illustrative and not restrictive. The present invention may be embodied in other ways without departing from the spirit and essential characteristics thereof.

Accordingly, it should be understood that all modifications falling within the spirit and scope of the present invention are covered by the appended claims.

[0034]

For example, only the X-Z plane shown in FIG. 6 may be used as the lane

in the embodiment of FIG. 5 to reduce the lane area, compared with that for two-dimensional electrophoresis.

In addition, the distribution of concentration in the depth (Z-axis) direction can be realized by wetting only one side of the substrate with a highly concentrated solution or applying a density gradient in the depth direction by means of centrifugation. This distribution can also be realized by stacking multiple layers of gel with different concentrations.

[0035]

If samples and markers are placed separately in the depth direction as shown in FIG. 7, it is possible to perform measurement using a compact electrophoresis system with all other conditions being the same as those of FIG. 6. In this case, the same fluorescence color may be used since lanes can be isolated in the depth direction by a confocal method.

When analyzing electrophoresis using a non-scanning confocal microscope, a sample may be positioned so that the aperture 61 of the confocal microscope is aligned with the sample position 62 or with part of the sample, as shown in FIG. 8. Consequently, it is possible to perform measurement with higher S/N ratios and without any adverse effect that may result when edges of the sample are measured.

[0036]

As the light source, either a single-grating or dual-grating light source may be used because they have the same effect.

[0037]

[Effect of the Invention]

As described above, the advantages offered by the present invention are as follows:

1) It is possible to easily realize highly precise polychrome electrophoresis using a compact system.

2) It is possible to realize three-dimensional electrophoresis using a compact system. In addition, large amounts of interrelated information can be acquired in a shorter length of time.

[Brief Description of the Drawings]

[FIG. 1]

FIG. 1 is a schematic block diagram showing one embodiment of a polychrome electrophoresis system in accordance with the present invention.

[FIG. 2]

FIG. 2 is a graph showing the distribution of wavelengths of excitation light and fluorescent light.

[FIG. 3]

FIG. 3 is a schematic view showing the arrangement of samples and markers.

[FIG. 4]

FIG. 4 is a schematic view showing a case where samples and markers are injected into the same lane.

[FIG. 5]

FIG. 5 is a schematic view showing a lane area when three-dimensional electrophoresis is conducted.

[FIG. 6]

FIG. 6 is a schematic view showing a case where a lane on each axis is

isolated.

[FIG. 7]

FIG. 7 is a schematic view showing a case where markers are arranged along the depth of samples.

[FIG. 8]

FIG. 8 is a schematic view showing the relationship between sample positions and apertures.

[FIG. 9]

FIG. 9 is a schematic view showing an example of a conventional electrophoresis system.

[FIG. 10]

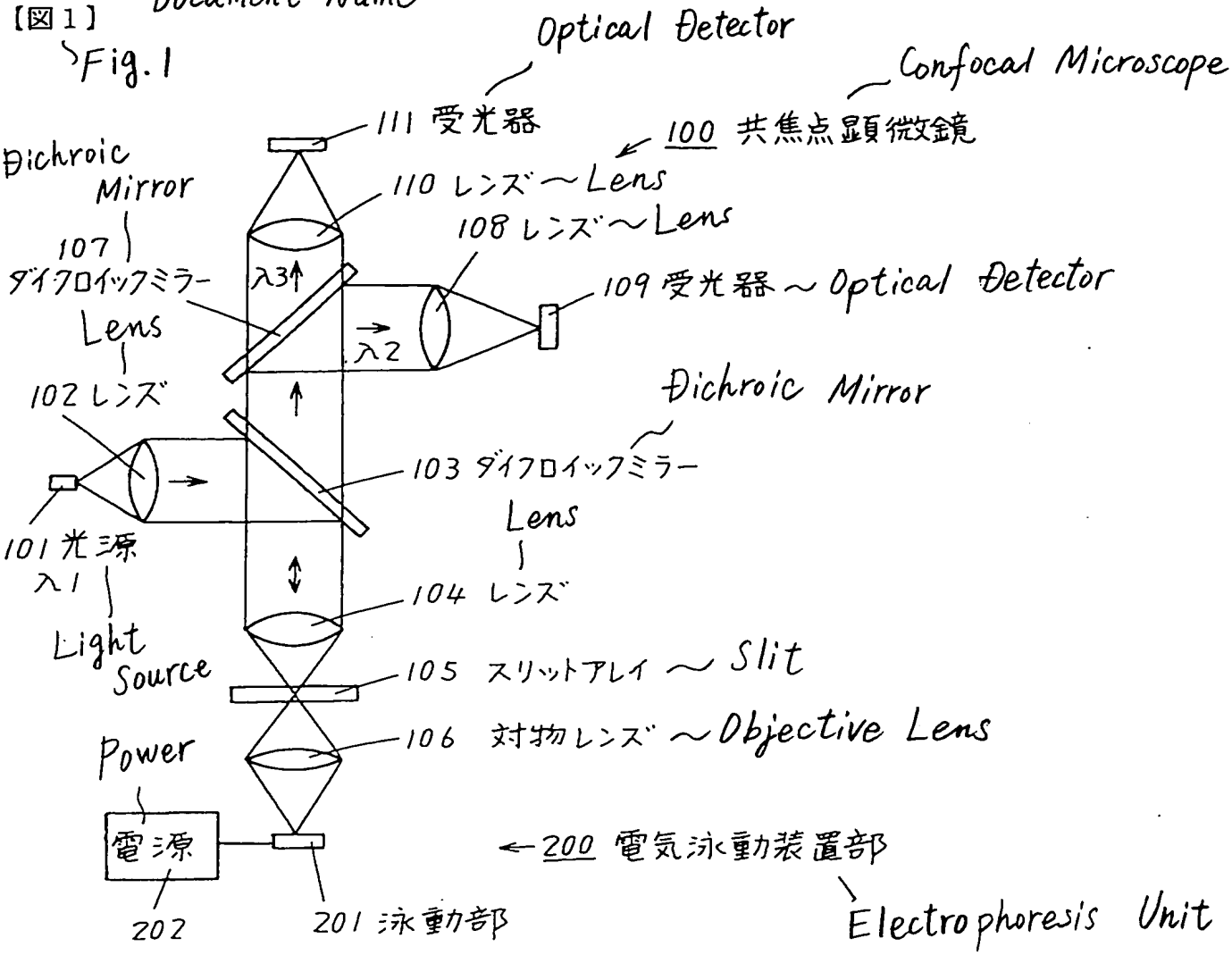
FIG. 10 is a schematic view showing a pattern of electrophoresis.

[Explanations of Letters or Numerals]

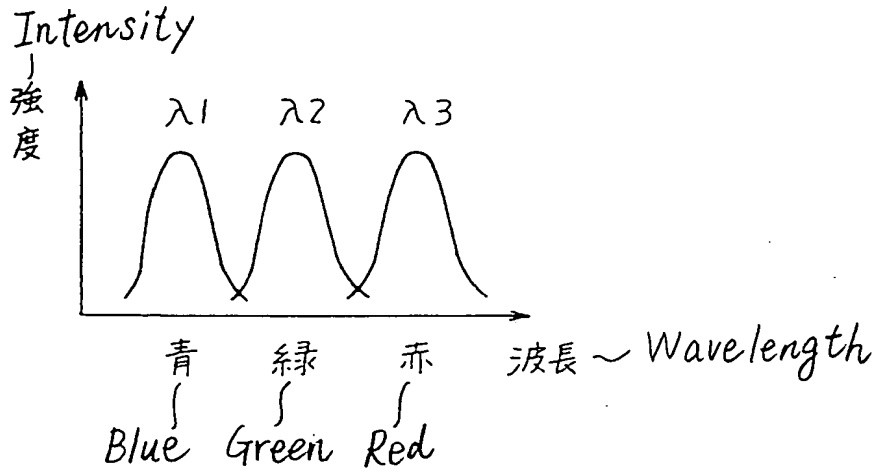
100	Confocal microscope
101	Light source
102, 104, 108, 110	Lenses
103, 107	Dichroic mirrors
105	Slit array
106	Objective lens
109, 111	Optical detectors
200	Electrophoresis unit
201	Lane area
202	Power unit

【書類名】　図面 ～ Drawings
Document Name

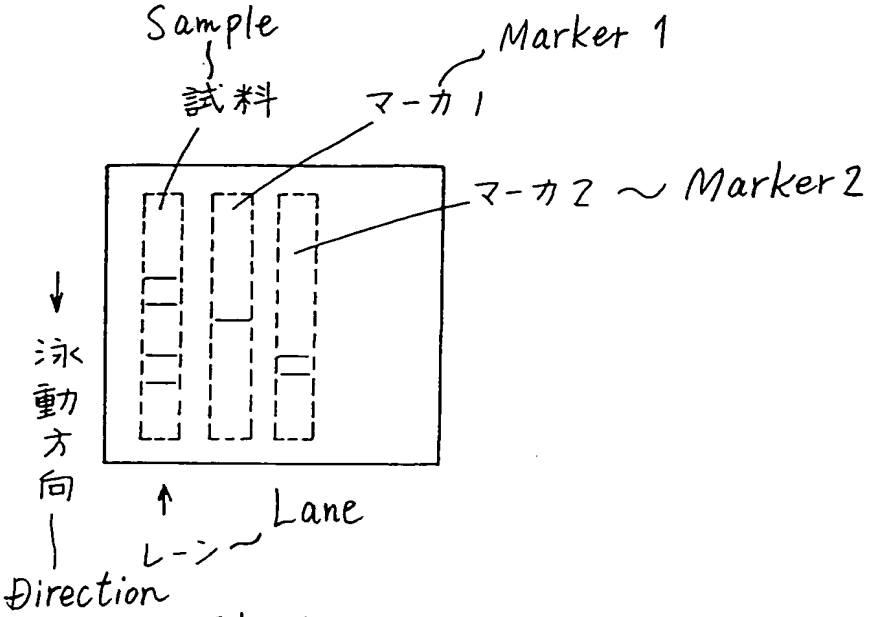
【図１】
Fig. 1



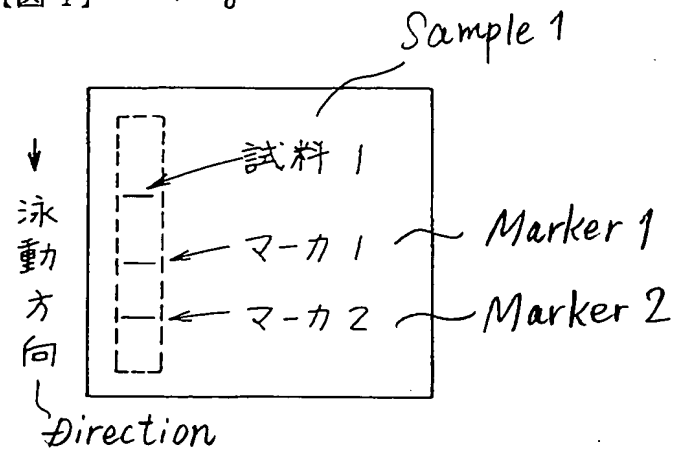
【図２】～ Fig. 2



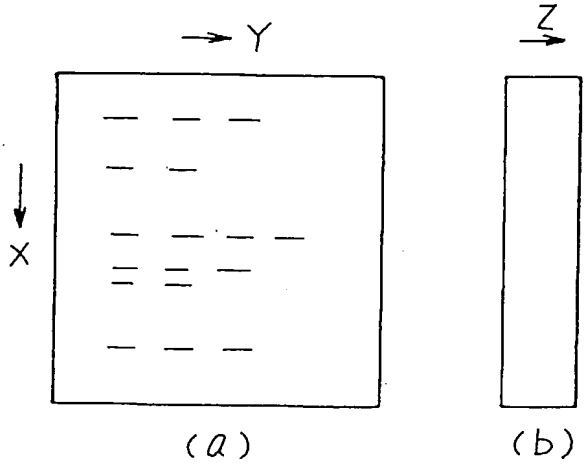
【図３】～Fig.3



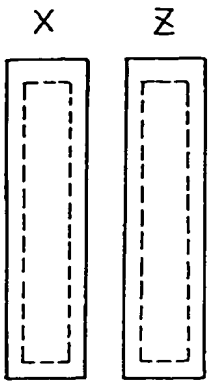
【図４】～Fig.4



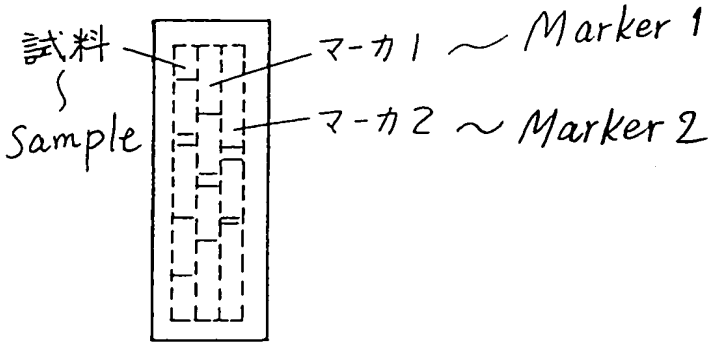
【図５】～Fig.5



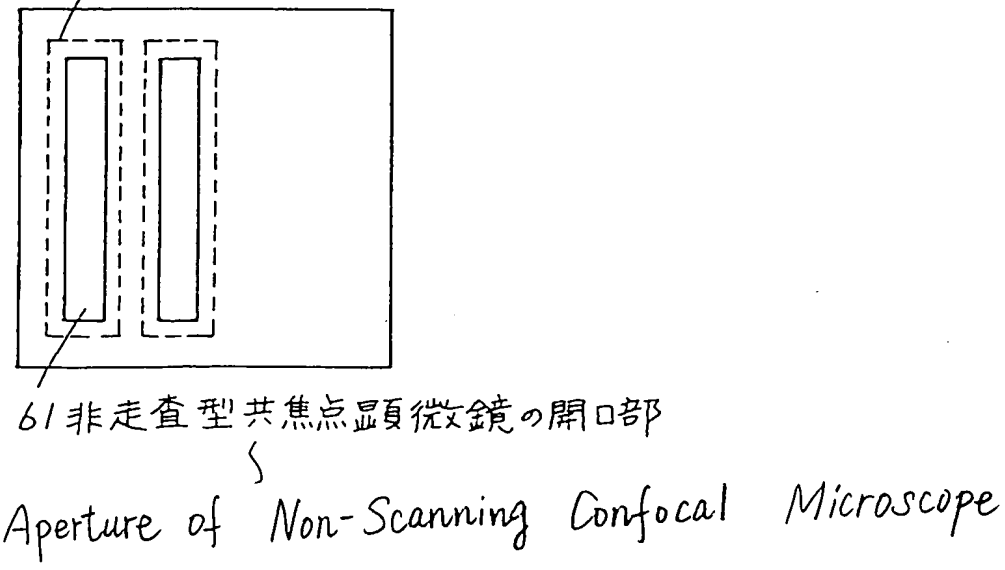
【図６】～Fig. 6



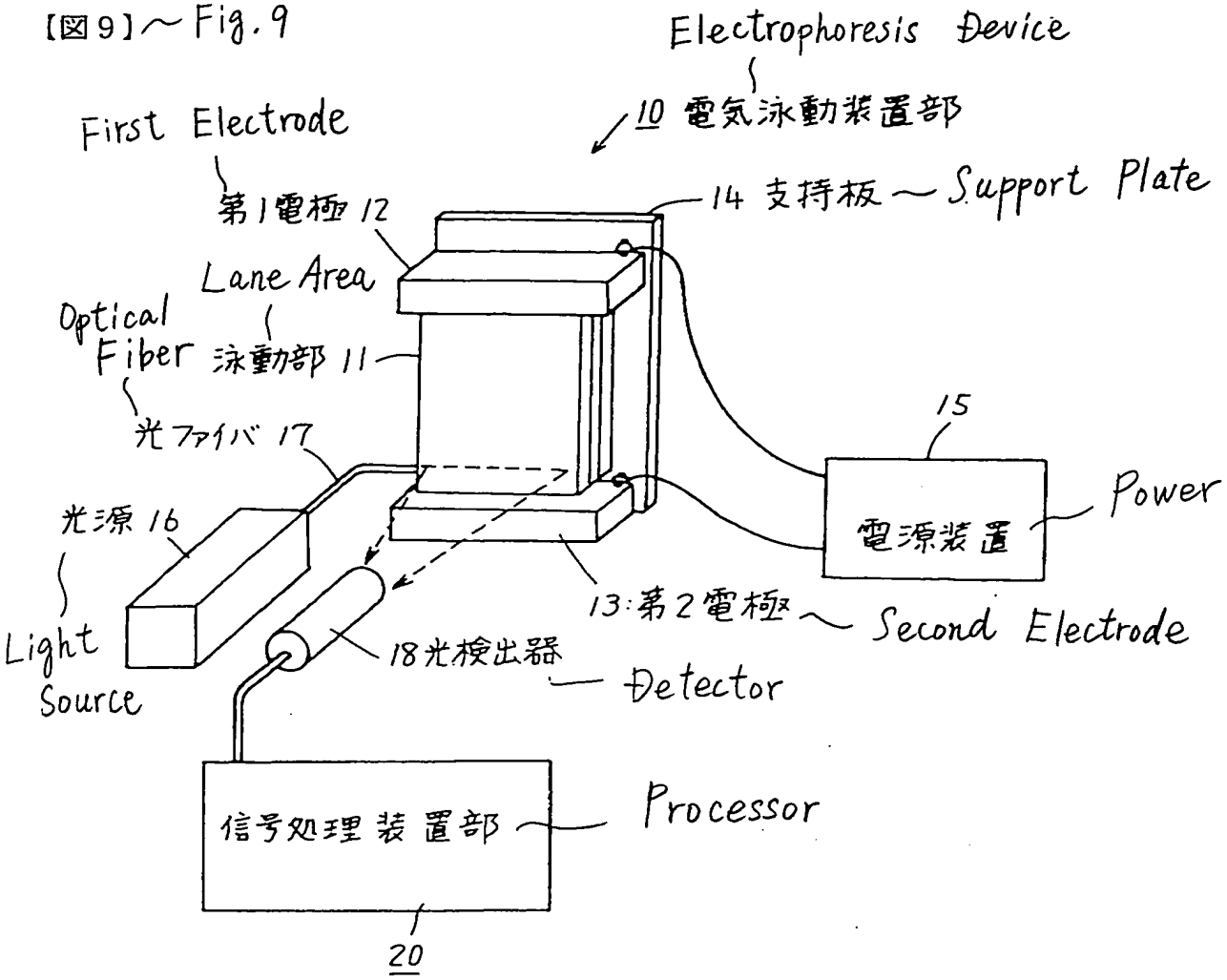
【図７】～Fig.7



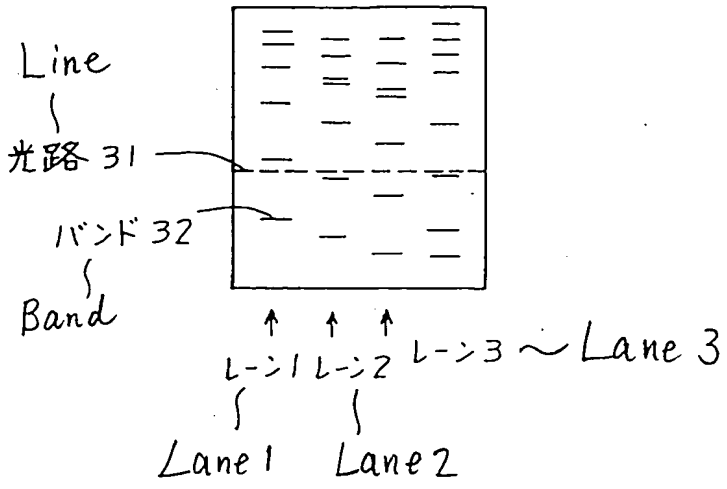
【図８】～Fig.8



【図９】～Fig. 9



【図１０】～Fig. 10



[Document Name] Abstract

[Abstract]

[Object of the Invention]

The object of the present invention is to provide an electrophoresis system which has a compact lane area, offers highly accurate electrophoretic patterns, and permits faster acquisition of large amounts of interrelated information.

[Means for Solving Problems]

An electrophoresis system for conducting electrophoresis of a sample marked with fluorescent coloring matter in a lane area so that a fluorescence pattern that is produced is read, comprising:

an electrophoresis unit for conducting electrophoresis by flowing a plurality of samples prepared by combining various types of target substance such as protein or DNA with different types of fluorescent coloring matter into the same lane of the lane area; and

a confocal scanner or a fluorescence imaging system wherein samples in the lane area are scanned with excitation light and polychrome fluorescence patterns of the samples produced by irradiating the excitation light are simultaneously detected through a plurality of filters having different transmission characteristics,

thereby allowing a plurality of electrophoretic patterns to be detected simultaneously.

[Chosen Drawing] FIG. 1